Application No.: 10/018,595 2 Docket No.: 03795/000J958-US0

## **AMENDMENTS TO THE CLAIMS**

1. (Currently amended) A bioreactor culture system for <u>mass</u> producing <u>mature</u> conifer somatic embryos, comprising:

a closed vessel;

a biomass immobilization matrix positioned in the closed vessel, said immobilization matrix having a vertical configuration;

a pump enabling an adjustment of a level of a liquid culture medium contained in the closed vessel, the level of liquid culture medium substantially submerging the immobilization matrix in a first initial flooding condition, and the level of liquid culture medium being equal to or lower than a lower end of the immobilization matrix in a subsequent maturation step of the conifer somatic embryos; and

a liquid culture medium spraying equipment for spraying liquid culture medium onto the biomass immobilization matrix to thereby irrigate said immobilized biomass <u>during said</u> maturation step.

- 2. (Original) The <u>culture</u> system of claim 1, further comprising a gas control equipment for controlling the concentration of oxygen in the gas phase of the closed vessel.
  - 3-5. (Cancelled)
- 6. (New) The culture system of claim 1, further comprising a means for periodical nutrient refreshment or replacement.
- 7. (New) The culture system of claim 2, wherein the gassing is at a controlled flow rate to maximize conifer somatic embryo production.
- 8. (New) The culture system of claim 1, wherein the closed vessel is equipped with a medium pumping port, a spray nozzle port for medium feeding and recirculation, a gas inlet and a gas outlet.

9. (New) The culture system of claim 1, wherein the immobilization matrix is made of sterilizable material.

- 10. (New) The culture system of claim 9, wherein the immobilization matrix comprises non-woven polyester fibers.
- 11. (New) The culture system of claim 1, wherein said closed vessel has a volume ranging from about 2 L to about 100L.
- 12. (New) The culture system of claim 1, wherein said immobilizing matrix has a vertical spiral configuration.
- 13. (New) The culture system of claim 1, wherein the conifer somatic embryos are selected from the group consisting of spruce, larch, pine, and hybrids thereof.
- 14. (New) The culture system of claim 13, wherein said spruce, larch, pine and hybrids thereof are selected from the group consisting of *Picea spp.*, *Picea glauca*, *Picea mariana*, *Picea abies*, *Picea rubens*, *Larix spp.*, *Larix decidua*, *Pinus spp.*, and hybrids thereof.
- 15. (New) The process of claim 14, wherein said conifer somatic embryos are selected cultivars thereof or genetically transformed.
- 16. (New) The culture system of claim 2, wherein said controlled gassing comprises an oxygen gas concentration maintained at about 21% for the first week of the maturation step and is dropped to about 4.2% thereafter.
- 17. (New) The culture system of claim 2, wherein said controlled gassing comprises an oxygen gas concentration maintained at about 21% during the whole maturation step.
- 18. (New) A process for the mass production of mature conifer somatic embryos in a bioreactor comprising:
- (a) inoculating a suitable culture medium in said bioreactor with conifer embryogenic tissues;

- (b) immobilizing said embryogenic tissues onto a biomass immobilizing matrix contained in said bioreactor, under initial flooding conditions thereof;
- (c) reducing the level of said culture medium in said bioreactor to a level such that only a lower end of said matrix, or less, remains immersed in said medium; and
- (d) subjecting said attached embryogenic tissues to a maturation step under controlled humidified conditions,

thereby enabling mass production of mature conifer somatic embryos.

- 19. (New) A process as defined in claim 18, wherein the immobilizing is carried-out while maintaining said matrix immersed in said culture medium, and a mixing of the liquid culture medium and the conifer embryogenic tissues is carried-out under low shear conditions until said embryogenic tissues attach to the immobilizing matrix and form an immobilized biomass.
- 20. (New) The process of claim 18, wherein said level of medium in (c) is reduced to a level below that of the biomass immobilizing matrix.
  - 21. (New) The process of claim 18, which further comprises:
- (a) installing a biomass immobilization matrix in a closed vessel of said bioreactor; and
- (b) sterilizing said biomass immobilization matrix and said closed vessel, prior to the inoculating step.
  - 22. (New) The process of claim 18, wherein the maturation step comprises:
    - (a) maintaining an immobilized, maturing biomass under sterile conditions; and
    - (b) spraying of a liquid medium over said immobilization matrix.
- 23. (New) The process of claim 18, further comprising removing most of said culture medium between said immobilizing of said tissues and said maturation step.
- 24. (New) A process for the mass production of mature conifer somatic embryos in a bioreactor comprising:

(a) installing a biomass immobilization matrix in a closed vessel of said bioreactor;

- (b) sterilizing said matrix and said closed vessel;
- (c) introducing a suitable liquid culture medium in said closed vessel to immerse said matrix;
- (d) adding an inoculum suspension of conifer embryogenic tissues to said liquid culture medium;
- (e) mixing said liquid culture medium and said conifer embryogenic tissues under low shear conditions until said embryonic tissues attach to said immobilizing matrix and form an immobilized, maturing biomass;
  - (f) removing most of said liquid culture medium;
  - (g) maintaining said immobilized, maturing biomass under sterile conditions; and
- (h) spraying of a residual or replacement liquid medium over said immobilization matrix,

thereby enabling mass production of mature conifer somatic embryos.

- 25. (New) The process of claim 18, further comprising periodical nutrient refreshment or replacement.
- 26. (New) The process of claim 24, further comprising periodical nutrient refreshment or replacement.
- 27. (New) The process of claim 18, further comprising gassing of said bioreactor at a controlled flow rate to maximize somatic conifer embryo production.
- 28. (New) The process of claim 24, further comprising gassing of said bioreactor at a controlled flow rate to maximize somatic conifer embryo production.
- 29. (New) The process of claim 18, further comprising harvesting and germinating the mature somatic embryos.

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- 30. (New) The process of claim 24, further comprising harvesting and germinating the mature somatic embryos.
- 31. (New) The process of claim 18, wherein said immobilizing matrix has a vertical spiral configuration.
  - 32. (New) A culture of conifer somatic embryos obtained from the process of claim 6.
- 33. (New) The culture of conifer somatic embryos of claim 32, wherein at least 60% of said mature somatic embryos are morphologically normal.
- 34. (New) The culture of conifer somatic embryos of claim 32, wherein at least 70% of said mature somatic embryos are morphologically normal.
- 35. (New) The culture of conifer somatic embryos of claim 32, wherein about 90% of said mature somatic embryos can germinate.
- 36. (New) The culture of conifer somatic embryos of claim 32, which comprises at least about 8000, mature somatic embryos per liter.
- 37. (New) The culture of conifer somatic embryos of claim 32, which comprises about 15 000 mature somatic embryos per liter.